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## Genomic selection for lucerne biomass yield in different stress-prone environments

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### Abstract

Climate change and reduced irrigation water emphasize the need for lucerne (*Medicago sativa* L.) varieties with improved yield under drought and saline conditions. Marker-based selection may offer opportunities, owing to low yield gains displayed by phenotypic selection. This study generated preliminary results on the ability of genomic selection to predict lucerne breeding values and the extent of genotype  $\times$  environment (GE) interaction for several stress-prone environments. Some 128 genotypes issued from an elite broadly-based reference population were genotyped via Genotyping-by-Sequencing, and phenotyped according to biomass yield of their half-sib progenies over a period ranging from five months to two years in four agricultural environments of Algeria, Argentina, Morocco and Tunisia featuring drought or salinity stress, and three environments of Italy with contrasting managed drought stress. AMMI analysis and estimates of genetic correlation revealed large GE interaction even across drought-prone environments. Genome-enabled predictive ability was mostly low albeit not quite unfavourable when compared with phenotypic selection opportunities. It tended to be higher under favourable conditions than under stress and in the last harvest, suggesting that it may increase for yield collected over a longer time span. Genomic selection was particularly convenient for selecting varieties with wide adaptation across stress conditions.

**Keywords:** adaptation, alfalfa, breeding, drought tolerance, yield, genomics, salinity

### Introduction

Reduced rainfall due to climate change, and reduced quantity and quality of irrigation water due to water demand for non-agricultural uses, emphasize the need for lucerne varieties with improved yield under drought and saline conditions. However, plant breeding is challenged by low rates of genetic yield gain for phenotypic selection (Annicchiarico *et al.*, 2015a) and large genotype  $\times$  environment (GE) interaction that emerged, *inter alia*, from results of lucerne varieties and landraces across sites of the western Mediterranean basin (Annicchiarico *et al.*, 2011). Specific-adaptation responses proved to be associated with different adaptive traits that are difficult to combine into novel varieties with moderately wide adaptation (Annicchiarico *et al.*, 2013). This adaptation target could be useful, however, to cope with the wide year-to-year climatic variation that features in many Mediterranean-climate locations.

Genotyping-by-Sequencing (GBS; Elshire *et al.* 2011) and other high-throughput techniques have facilitated the application of genomic selection to plants. For species bred as synthetic varieties, the prediction of breeding values could conveniently rely on genomic models developed from genotyping data of candidate parent plants and phenotyping data of their half-sib progenies (Annicchiarico *et al.*, 2015a). A pioneering study for lucerne biomass yield of two reference populations in the absence of marked drought or salinity stress revealed the distinct advantage of GBS-based genomic selection over phenotypic selection according to predicted yield gains per unit time (Annicchiarico *et al.*, 2015b). This study aimed to verify the ability of genomic selection to predict lucerne breeding values for a range of environments mostly subjected to severe drought or salinity stress, and to assess concurrently the extent

of GE interaction across stress environments, for biomass yield data that were preliminarily available from a multi-environment phenotyping assessment.

## Materials and methods

Some 128 genotypes from a broadly-based Mediterranean reference population underwent GBS using the Elshire *et al.* (2011) protocol with modifications as described in Annicchiarico *et al.* (2015b). Their half-sib progenies were phenotyped in densely planted micro-plot trials including three to five replicates performed in three managed environments (MS) of northern Italy with contrasting drought-stress level, two drought-prone rain-fed sites of Algeria (Alger) and Argentina (Santiago del Estero), one drought-prone Moroccan site with supplemental irrigation (Marrakech), and a Tunisian site (Médénine) irrigated with moderately saline (9.37 dS/m) water. Dry matter yield was recorded over five months in moisture-favourable MS, and over a period of eleven months to two years elsewhere. GE interactions for yield of the half-sib progenies across stress environments were modelled by Additive Main effects and Multiplicative Interaction (AMMI) analysis (Gauch *et al.*, 2008), analysing  $\log_{10}$ -transformed data to compensate for heterogeneity of genotypic variance across sites. SNP calling held two homozygote classes *AAAA* and *aaaa*, and one heterozygote class pooling *AAAA*, *AAaa* and *Aaaa* variants. We retained for analyses 9,269 SNP markers with less than 20% missing data across genotypes, imputing missing data by the KNNI method. Genomic predictions were assessed by Ridge Regression BLUP and Support Vector Regression with linear kernel.

## Results and discussion

Nominal yields (i.e. AMMI-modelled yields from which the site main effect was subtracted) of nine top-yielding half-sib progenies as a function of the first GE interaction principal component (PC 1) score of the stress-prone environments revealed large GE interaction of cross-over type (Figure 1). PC 1 indicated distinct half-sib progeny responses in Marrakech, as confirmed by genetic correlations (where this site showed nil or negative correlation with the other environments). GE interaction PC 2 was also significant ( $P < 0.001$ ). The genetic correlations of the agricultural environments among themselves or with MS environments never exceeded 0.30, except for Médénine with moisture-favourable MS ( $r_g = 0.59$ ).

The two genomic selection models exhibited similar predictive ability. Genomic predictions were fairly high for moisture-favourable MS, and poor for the stress-prone sites of Alger, Médénine and Santiago del Estero (Table 1). The possibility to reach better predictions using future data for a longer time span was supported by better predictions obtained for the last harvest in Alger and Médénine. One possible reason for less accurate predictions in stress environments was their trend towards higher experiment

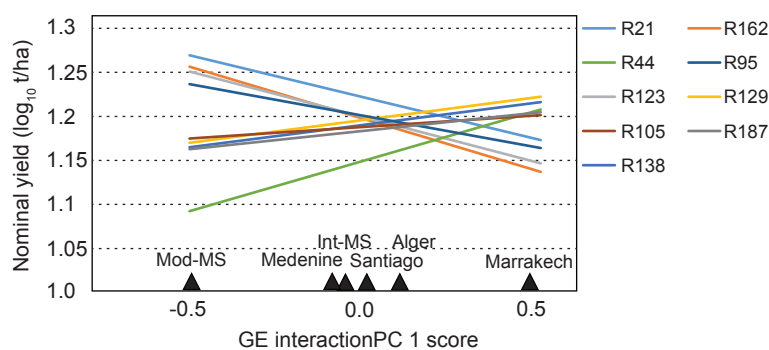


Figure 1. AMMI-1 nominal dry-matter yield of nine top-yielding lucerne half-sib progenies across moderate-drought or intense-drought managed stress environments (MS) and four agricultural environments.

Table 1. Assessment period and mean total biomass yield of managed-stress environments (MS) of Italy and agricultural environments, and predictive ability of genomic selection for lucerne breeding values (as correlation of modelled and observed data over 100 ten-fold stratified cross-validations for one top-predictive model).

Test environment	Period (month/year)	Mean yield (t ha <sup>-1</sup> )	Predictive ability	
			Total yield	Last harvest yield <sup>1</sup>
MS, intense drought	4/2014 – 4/2016	14.11	0.12	0.22
MS, moderate drought	10/2012 – 5/2014	10.11	0.18	0.10
MS, moisture-favourable	6/2012 – 10/2012	7.62	0.36	–
Alger (Algeria)	12/2015 – 12/2016	8.53	0.05	0.12
Marrakech (Morocco)	10/2015 – 10/2016	12.56	0.19	0.23
Médenine (Tunisia)	9/2015 – 11/2016	38.64	0.05	0.13
Santiago del Estero (Argentina)	12/2015 – 11/2016	13.43	0.10	–

<sup>1</sup> Excluding environments with relatively short experiment duration.

error than favourable environments. However, the value of genomic selection ought to be assessed in relation to opportunities offered by phenotypic selection as determined by narrow-sense heritability, selection cycle duration, and selection intensity (which is affected in turn by evaluation costs). In this respect, even a prediction ability around 0.15 would be sufficient to grant some advantage to genomic selection for lucerne yield relative to phenotypic selection (Annicchiarico *et al.*, 2017).

The genomic prediction ability for progeny mean yield over stress environments (computed from relative yields in each environment) was 0.14, which is promising for selecting widely-adapted varieties. Phenotypic selection would be less convenient in this prospect, owing to high evaluation costs and low heritability caused by high GE interaction.

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